HEK-Blue™ KD-TLR5 Cells

TLR5-deficient IL-1β Reporter Cells

Catalog code: hkb-kdtlr5

https://www.invivogen.com/hek-blue-kd-tlr5

For research use only

Version 19F13-MM

PRODUCT INFORMATION

Contents:

- **1 vial of HEK-Blue™ KD-TLR5 Cells** (3-7 x 10^6 cells)
- **1 ml of Zeocin™** at 100 mg/ml. Store at 2-8 °C or at -20 °C.*
- **1 ml of puromycin** at 10 mg/ml. Store at 2-8 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

- **1 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20 °C. QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C.

Handling Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

Note: ***Avoid freezing cells upon receipt*** *as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- TLR5 knockdown is verified by functional assays and qRT-PCR.
- SEAP reporter activity has been validated using functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees with the terms and conditions of all applicable Limited Use Label Licenses.

For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

PRODUCT DESCRIPTION

HEK-Blue™ KD-TLR5 cells are designed to monitor bioactive IL-1β secreted by THP-1 cells upon flagellin-induced NLRC4 activation. HEK-Blue™ KD-TLR5 cells are derived from the HEK293 cell line, which endogenously expresses TLR5 and the IL-1β receptor (IL-1R). This cell line features an NF-κB-inducible SEAP reporter gene and was engineered to knock-down the expression of TLR5 to avoid activation of NF-κB upon flagellin-induced TLR5 stimulation. The knockdown of TLR5 permits the analysis of flagellin specifically for its NLRC4 stimulating activity.

Binding of IL-1β to IL-1R initiates a signaling cascade leading to the activation of NF-κB and the subsequent production of SEAP. Detection of SEAP in the supernatant of HEK-Blue™ KD-TLR5 cells can be readily assessed using QUANTI-Blue™ Solution.

HEK-Blue™ KD-TLR5 cells are resistant to the selective antibiotics Zeocin™ and puromycin.

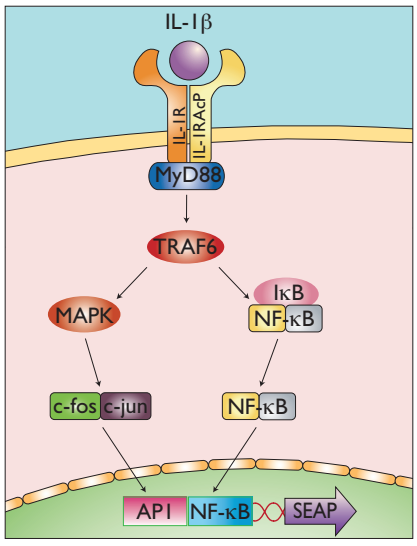

Figure 1: IL-1β-induced NF-κB signaling pathway.

TECHNICAL SUPPORT

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FAQ Any questions about our cell lines? Visit our FAQ page.

InvivoGen www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ KD-TLR5 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require **Biosafety level 2** according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, in Germany HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56°C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO
- **Test Medium:** DMEM 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin™, Puromycin and Zeocin™**

Required Selection Antibiotics

- Puromycin and Zeocin™

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% (v/v) ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 1000-1200 RPM (RCF 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the vial contents to a T-25 culture flask containing 5 ml of growth medium.

Note: To avoid excessive alkalinity of the medium during recovery of the cells, place the tissue culture flask containing the growth medium into the incubator for at least 15 minutes prior to the addition of the vial contents.

7. Place the culture at 37°C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10^6 cells/ml in freezing medium prepared extemporaneously with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Aliquot 1 ml cells into cryogenic vials.
3. Place vials in a freezing container and store at -80°C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell maintenance

1. Maintain and subculture the cells n growth medium supplemented with 1 µg/ml puromycin and 100 µg/ml Zeocin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached, detach the cells in presence of PBS by tapping the flask or by using a cell scraper. Do not let the cells grow to 100% confluency.

Note: The response of HEK-Blue™ KD-TLR5 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ KD-TLR5 cells.

Cell Handling Recommendations

To ensure the best results:
- Use HEK-Blue™ KD-TLR5 cells with less than 20 passages.

APPLICATION

HEK-Blue™ KD-TLR5 cells are designed to monitor bioactive IL-1β secreted by THP-1 cells upon flagellin-induced NLRC4 activation. Levels of IL-1β secreted into the supernatant of THP-1 cells can be monitored using the HEK-Blue™ KD-TLR5 cell line. THP1-NLRC4 and HEK-Blue™ KD-TLR5 cell lines can be used sequentially (option 1) or co-cultured to save time (option 2).

DETECTION OF IL-1β IN THP-1 SUPERNATANTS

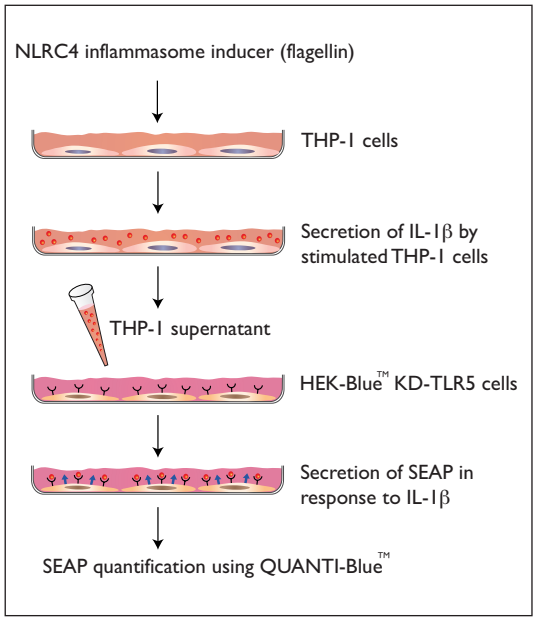

Figure 2. THP-1/HEK-Blue™ IL-1β Assay

• Option 1: Sequential culture of THP1-NLRC4 & HEK-Blue™ KD-TLR5 cells

Activation of THP1-NLRC4 cells

THP1-NLRC4 cells are grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 µg/ml Normocin™, and Pen-Strep (100 U/ml-100 µg/ml). THP1-NLRC4 cells are grown in suspension to a density of 1 x 10^6 cells/ml in tissue culture flasks.

Day 1
1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control (such as FLA-BS Ultrapure, 200 ng/ml) in one well.
3. Prepare a THP1-NLRC4 cell suspension at 1 x 10^6 cells/ml and add 180 µl of this cell suspension per well of a 96-well plate (~ 2 x 10^5 cells/well).
4. Incubate overnight at 37°C in 5% CO_2.

Detection of IL-1β by HEK-Blue KD-TLR5 cells

HEK-Blue™ KD-TLR5 cells are grown in DMEM supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 µg/ml Normocin™, and Pen-Strep (100 U/ml-100 µg/ml).

Day 2
1. Prepare HEK-Blue™ KD-TLR5 cell suspension: wash cells with pre-warmed PBS, detach cells by tapping the flask, resuspend cells in fresh growth medium and prepare a cell suspension at ~ 3 x 10^5 cells/ml. *Note: The response of HEK-Blue™ KD-TLR5 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ KD-TLR5 cells.*
2. Add 50 µl of activated THP1-NLRC4 cell supernatant per well of a flat-bottom 96-well plate.
3. In separate wells, add 50 µl of recombinant human IL-1β at 0.25 µg/ml, as the positive control, and 50 µl of growth medium, as a negative control.
4. Add 150 µl of HEK-Blue™ KD-TLR5 cell suspension (~ 5 x 10^4 cells) per well.
5. Incubate overnight at 37°C in 5% CO_2.

Day 3
6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
7. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of induced HEK-Blue™ KD-TLR5 cells supernatant.
9. Incubate the plate at 37°C for 1-6 hours.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Option 2: Co-culture of THP1-NLCR4 & HEK-Blue™ KD-TLR5 cells

Activation of THP1-NLRC4 cells with concurrent detection of IL-1β by HEK-Blue KD-TLR5 cells

Day 1
1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control (such as FLA-BS Ultrapure, 200 ng/ml) in one well.
3. Prepare a THP1-NLRC4 cell suspension in IMDM, 10% heat-inactivated FBS at 2 x 10^6 cells/ml and add 90 µl of this cell suspension, per well of a 96-well plate (~ 2 x 10^5 cells/well).
4. Prepare a HEK-Blue™ KD-TLR5 suspension in IMDM, 10% heat-inactivated FBS at 5 x 10^5 cells/ml and add 90 µl of this cell suspension per well of a 96-well plate (~ 5 x 10^4 cells/well).
5. Incubate overnight at 37°C in 5% CO_2.

Day 2
6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
7. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of cells supernatant.
9. Incubate the plate at 37°C for 1-6 hours.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
Recombinant human IL-1β	Recombinant cytokine	rcyec-hil1b
Normocin™	Antimicrobial agent	ant-nr-1
Puromycin	Selection antibiotic	ant-pr-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
THP1-NLRC4	Inflammasome test cells	thp-nlrc4
Zeocin™	Selection antibiotic	ant-zn-1
NLRC4 Inflammasome inducer:		
FLA-BS Ultrapure	Flagellin from *B. subtilis*	tlrl-pbsfla
FLA-PA Ultrapure	Flagellin from *P. aeruginosa*	tlrl-pafla
FLA-ST Ultrapure	Flagellin from *S. typhimurium*	tlrl-pstfla

QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2

https://www.invivogen.com/quanti-blue

For research use only

Version 19F11-MM

PRODUCT INFORMATION

Contents

QUANTI-Blue™ Solution is available in two pack sizes:

- **rep-qbs** containing 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer to prepare 500 ml of QUANTI-Blue™ Solution sufficient for 25 x 96-well plates (standard procedure) or 20 x 1536-well plates (HTS screening)
- **rep-qbs2** containing 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer to prepare 1 liter of QUANTI-Blue™ Solution sufficient for 50 x 96-well plates (standard procedure) or 40 x 1536-well plates (HTS screening)

Required Material (not provided)

- Sterile water
- Sterile screw cap tube, glass bottle or flask

Storage and Stability

- Store QB reagent and QB buffer at -20 °C. Product is stable for 1 year at -20 °C when properly stored.
- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Protect QUANTI-Blue™ from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (including pH, solubility).
- Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION

QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP.

Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters.

FEATURES AND ADVANTAGES

- **Requires small samples of cell supernatants** - 20 µl is sufficient.
- **No need to process samples** - Preparation of cell lysates or heating of samples is not required.
- **Determine secreted AP activity without disturbing cells** - The same cell cultures can be repeatedly sampled for kinetic studies.
- **Assay can be completed in 30 min** - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™ Solution.
- **Wide dynamic range allows to detect low and high levels of AP** - No need to perform multiple sample dilutions.
- **Highly sensitive for quantitative measurement** - Higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity.
- **Extremely simple to use** - 1) Prepare solution with water, 2) add sample to detection reagent, 3) incubate at 37 °C, and 4) assess AP activity.

METHODS

QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

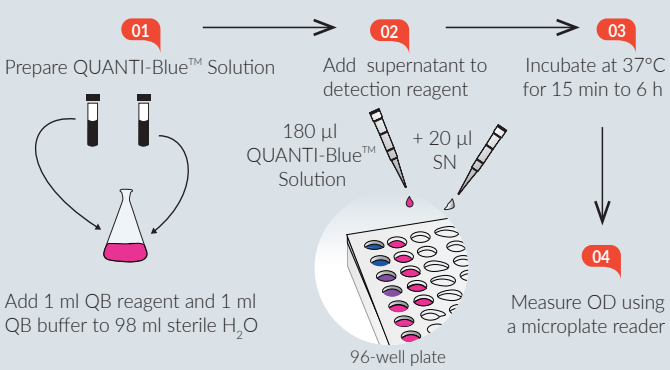

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

*Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does **not** exceed 5 minutes.*

1. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water in a sterile glass bottle or flask.
2. Mix well by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37 °C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

TECHNICAL SUPPORT
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B. High Throughput Screening (HTS) procedure

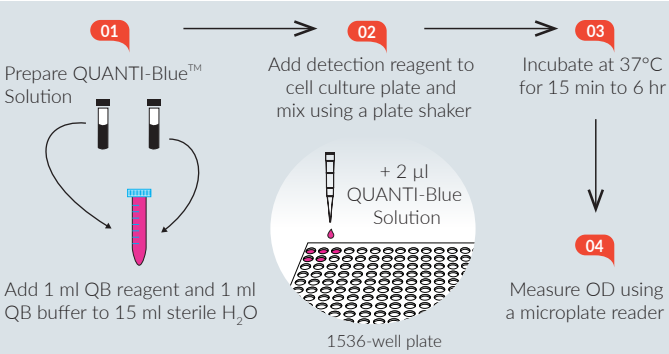

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling.
Ensure QB reagent and QB buffer are completely thawed before use.
Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does ***not*** *exceed 5 minutes.*

1. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed **5 µl** per well. Incubate cells with test compounds for the desired period of time.
2. Prepare 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile water in a 50 ml screw cap tube.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
5. Dispense **2 µl of QUANTI-Blue™ Solution** to the wells containing ≤5 µl of cell culture in a 1536-well plate.
6. Mix using a plate shaker.
7. Incubate at 37°C for 15 min to 6 h.
8. Measure OD at 620-655 nm.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo^R)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit
https://www.invivogen.com/reporter-cells

TECHNICAL SUPPORT
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